Standard Operating Procedure for the Determination of Chlorinated Herbicides in Water

1.0 Scope and Application

1.1 This is a gas chromatographic (GC) method and applicable for the extraction and determination for the listed analytes (Sect. 1.2). This method is for the extraction of the analytes in water. This method should be used by, or under the supervision of an experienced analyst. The analyst should be skilled in liquid-liquid extractions, derivatization procedures, and the use of the GC and interpretation of gas chromatograms.

1.2 Target compounds that can be measured using this method include the following:

Analyte	CAS#
Acifluorfen	50594-66-6
Bentazon	25057-89-0
Bromoxynil	1689-84-5
4-Chloro-o-tolyoxyacetic acid (MCPA)	94-74-6
2,4-D	94-75-7
2,4-DB	94-82-6
Dacthal (Chlorthal)	1861-32-1
Dalapon	75-99-0
Dicamba	1918-00-9
3,5-Dichlorobenzoic Acid (3,5-DCBA)	51-36-5
Dichlorprop	120-36-5
Dinoseb	88-85-7
Diclofop (Hoelon)	40843-25-2
Pentachlorophenol (PCP)	87-86-5
Picloram (Tordon)	1918-02-01
2,4,5-T	93-76-5
2,4,5-TP (Silvex)	93-72-1

1.3 This method is applicable to the determination of the target analytes over the ranges of 0.02-12.5ug/L. The method detection limits (MDLs) are determined by the procedure in 40 CFR Part 136, Appendix B.

1.4 The following compounds may require some special attention when being determined by this method:

1.4.1 2,4-DB co-elutes with Dinoseb on the RTX-5ms column. Since the RTX-5 column is the confirmation column this problem can be corrected by

analyzing the sample on the GC/MS system.

1.4.2 The analytes are extracted as carboxyl acids, which breakdown in the injection port of the GC. To correct this problem the analytes are derivatized with diazomethane before analysis.

2.0 Summary of Method

2.1 One liter of sample is adjusted to pH 12 with sodium hydroxide and shaken for one hour to hydrolyze derivatives. A solvent wash can be done at this time to remove extraneous organic materials. The sample is then acidified, and the chlorinated acids are extracted with dichloromethane. The extracted acids are then converted to their methyl esters using diazomethane. Excess diazomethane is destroyed and the esters are determined by GC/ECD.

2.2 A Florisil cleanup procedure is provided to aid in the elimination of interferences that may be encountered.

3.0 Definitions

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

3.1 GC/ECD - Gas chromatograph/electron capture detector

3.2 Reagent water - Reagent water is defined as water that is reasonably free of contamination that would prevent the determination of any analyte of interest. A Millipore or Barnstead water system or its equivalent may be used to generate deionized reagent water. Distilled water that has been passed through granular charcoal may also be suitable. Reagent water is monitored through analysis of the laboratory reagent blank (LRB.)

3.3 Quality Control Sample (QCS) - A solution of method analytes of known concentration which is used to fortify an aliquot of reagent water or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.4 Laboratory Fortified Blank (LFB) - An aliquot of reagent water to which known

quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and it purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5 Laboratory Reagent Blank (LRB) - An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 Laboratory Fortified Matrix (LFM) - Spiked Sample - An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.7 Laboratory duplicates - Two aliquots, LD1 and LD2, of the sample designated as such in the laboratory. Each aliquot is extracted and analyzed separately with identical procedures. Analysis of LD1 and LD2 indicate the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.8 Field duplicates (FD1 and FD2) - Two separate samples collected at the same time and placed under identical circumstances and treated the same throughout field and laboratory procedures. Analysis of FD1 and FD2 gives a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.9 Target Compound - An analyte or compound listed in section 1.2.

3.10 Surrogate analyte - A pure analyte that is chemically similar to the target compounds, but is not expected to occur in an environmental sample. It is added to a sample aliquot in a known amount before extraction and is measured with the sample procedures used to measure other sample components. The purpose of the surrogate is to monitor method performance with each sample.

3.11 Spiking Solution - A mixture of the analytes listed in section 1.2. These compounds are added to the LFB and LFM in known amounts. The spiking

solution is added before extraction to measure any effects of the matrix and laboratory procedures on the analytes and surrogates.

3.12 Stock Standard Solution (SSS) - A concentrated solution of one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.13 Primary dilution standard solution - A solution of several analytes prepared in the laboratory from SSS and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.14 Calibration Standards (CAL) - A solution prepared from the primary dilution standard solution or stock standard solution and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.15 Tube spike and surrogate - Laboratory performance check sample - a solution of method analytes and surrogate in solvent that is used to evaluate the performance of the instrumental system with respect to a defined set of method criteria.

3.16 Material Safety Data Sheet (MSDS) - Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill, and handling precautions.

4.0 Interferences

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by analyzing laboratory reagent blanks as described in section 9.0.

4.1.1 Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by throughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap water, dilute acid, and reagent water. Drain dry. Thorough rinsing with acetone and hexane will help to eliminate any contaminants that may remain after washing. After drying and rinsing, store glassware in a clean environment free of all potential contaminants. Store glassware inverted or capped with aluminum foil.

4.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Each new bottle of solvent should be analyzed before use. An interference free solvent is a solvent containing no peaks representing a concentration level above the reporting level at the retention times of the analytes of interest. Purification of solvents by distillation in all-glass systems my be required. WARNING: When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous. Also, when a solvent is purified, preservatives added by the manufacturer are removed, thus potentially reducing the shelf-life.

4.2 Analytical bias may result from discrimination at the GC inlet. This can be minimized by optimizing the inlet configurations and injection technique.

4.3 The acidic form of the analytes are strong organic acids which react readily with alkaline substances and can be lost during sample preparation. Glassware and glass wool must be acid-rinsed with 1N hydrochloric acid prior to use to avoid analyte losses due to adsorption.

4.4 Organic acids and phenols, especially chlorinated compounds, cause the most direct interferences with the determination. Alkaline hydrolysis and subsequent extraction of the basic sample removes many chlorinated hydrocarbons and phthalate esters that might otherwise interfere with the electron capture analysis. However, most water samples are already free of these interferences so the basic extraction is only done on the dirtiest samples.

4.5 Interferences by phthalate esters can pose a major problem in pesticide analysis when using the ECD. These compounds generally appear in the chromatogram as large peaks. Common flexible plastics contain varying amounts of phthalates, that are easily extracted or leached during laboratory operations. Cross contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalate can best be minimized by avoiding the use of plastics in the laboratory. Exhaustive purification of reagents and glassware may be required to eliminate background phthalate contamination.

4.6 It is important that samples and working standards be contained in the same solvent. The solvent for working standards must be the same as the final solvent used in sample preparation. If this is not the case, chromatographic comparability of standards to sample may be affected.

4.7 Matrix interferences may be caused by contaminants that are co-extracted from the sample. Also, note that all analytes listed in section 1.2 are not resolved from each other on any one column. In other words one analyte of interest may be an interferent for another analyte of interest. The extent of matrix interferences will vary considerably from source to source, depending upon the water sampled. Positive analyte identification should be confirmed using the confirmation column.

5.0 Safety

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of MSDSs should also be made available to all personnel involved in the chemical analysis.

5.2 DIAZOMETHANE - A toxic carcinogen which can explode under certain conditions. The following precautions must be followed:

5.2.1 Use only in a well ventilated hood - do NOT breathe vapors.

5.2.2 Use a safety screen.

5.2.3 Use mechanical pipetting aides.

5.2.4 Do not heat above 90°C - **EXPLOSION** may result.

5.2.5 Avoid grinding surfaces, ground glass joints, sleeve bearings, glass stirrers- **EXPLOSION** may result.

5.2.6 Store away from alkali metals - **EXPLOSION** may result.

5.2.7 Solutions of diazomethane decompose rapidly in the presence of solid materials such as copper powder, calcium chloride, and boiling chips.

5.2.8 The diazomethane apparatus used in the esterification procedures (Sect. 11.4) produces micromolar amounts of diazomethane to minimize safety hazards.

5.3 WARNING - When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous.

6.0 Equipment and Supplies

NOTE: Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

6.1 Sample bottle - Borosilicate, 1-4L volume, fitted with screw caps lined with TFE-fluorocarbon. Protect samples from light. The container must be washed and dried as described in section 4.1.1 before use to minimize contamination.

6.2 Glassware

6.2.1 Separatory funnel - 2000-ml, with TFE-fluorocarbon stopcocks, ground glass or TFE-fluorocarbon stoppers.

6.2.2 Tumbler bottle - Teflon 1.7-L with TFE-fluorocarbon lined screw cap.

6.2.3 Zymarck Turbo Vap ll concentration system or equivalent to concentrate the samples to 1 ml after extraction. All associated glassware should be washed using procedures described in section 4.1.1.

6.2.4 Vials - glass, graduated 5-15mL capacity with TFE-fluorocarbon lined screw caps.

6.2.5 Disposable pipets - 5ml capacity (13-678-20D or equivalent)

6.3 Tumbler - Capable of holding tumbler bottles and tumbling them end-over-end at 30 turns/min.

6.4 Balance - Analytical, capable of accurate weighing to the nearest 0.01g.

6.5 Diazomethane Generator - Assemble from two 20x150mm test tubes, two Neoprene rubber stoppers, and a source of nitrogen as shown in Figure 1. The diazomethane collector is cooled in a cooled vessel.

6.6 Glass Wool - Acid washed and heated at 450°C for 4 hours.

6.7 Gas Chromatograph - Hewlett Packard 5890 Gas Chromatograph with data system

6.7.1 The GC must be capable of temperature programming and be equipped with a splitless injector.

6.7.2 GC supplies including injection port liners, ferrules, syringes, etc.

6.7.3 The gas chromatograph must be equipped with an electron capture detector.

6.7.4 An autoinjector is recommended for improved precision of analysis.

6.7.5 The interfaced data system is the LabSystems Xchrom or an equivalent system which allows for instrument control, data acquisition, storage, retrieval, and calculations of results. (See system manual for details).

6.7.6 Capillary column 30 meters long, 0.25mm ID, 0.25 micron film thickness or equivalent. Both columns use the same oven program and injector port. The injector temperature is 235°C. Both detectors are at 300°C. The oven is initially at 50°C held for 1 minute, then ramped to 226°C at 10C/min and held 12 minutes, the final ramp is to 286°C at 15C/min and held for 10 minutes.

6.7.6.1 Primary column - Restek RTX-50ms or equivalent column

6.7.6.2 Confirmation column - Restek RTX-5ms or equivalent column

7.0 Reagents and standards

7.1 Acetone, hexane, methanol, methylene chloride, MTBE, and ethyl ether-Optima grade, or nanograde, or distilled in glass, or in other words the highest purity to reduce any interference problems. The residue grade solvents are flammable and stored in an appropriate flammable storage area. Reagent grade inorganic chemicals shall be used in all tests.

7.2 Sodium Sulfate, granular, anhydrous, ACS grade - May be heat treated in a shallow tray at 450°C for a minimum of 4 hours to remove interfering organic

substances. Acidify by slurrying 100g sodium sulfate with enough ethyl ether to just cover the solid. Add 0.1mL concentrated sulfuric acid and mix thoroughly. Remove the ether under vacuum. Mix 1g of the resulting solid with 5mL of reagent water and measure the pH of the mixture. The pH must be below pH 4. Store at 130°C.

7.3 Sodium Hydroxide (NaOH), pellets - ACS grade

7.3.1 NaOH, 6N - Dissolve 216g NaOH in 900mL reagent water.

7.4 Sulfuric Acid, concentrated - ACS grade

7.5 Potassium Hydroxide (KOH), pellets - ACS grade

7.5.1 KOH, 37% - Dissolve 37g KOH pellets in reagent water and dilute to 100mL.

7.6 Carbitol (Diethylene glycol monoethyl ether) - ACS grade. Available from Aldrich Chemical company.

7.7 Diazald, ACS grade - Available from Aldrich chemical company

7.8 Diazald solution - Prepare a solution containing 5g Diazald in 50mL of a 50:50 by volume mixture of ethyl ether and Carbitol. This solution is stable for 3 months when stored at 4°C in an amber bottle with a Teflon-lined screw cap.

7.9 Sodium Chloride (NaCl), Crystal, ACS grade - Heat treat in a shallow tray at 450°C for a minimum of 4 hours to remove interfering organic substances.

7.10 2,4-Dichlorophenylacetic acid (2,4-DCAA) - 99% purity, for use as surrogate standard. Available from Chemservice and Aldrich Chemical Co. 2,4-DCAA must be in the acidic form.

7.10.1 125ug/mL: solution is used as surrogate: Prepare by pipetting 625uL of stock solution (2000ug/mL) into a 10mL volumetric flask. Fill the flask to mark with MTBE.

7.11 Reagent water -Reagent water is defined as water that is reasonably free of contamination that would prevent the determination of any analyte of interest. A Millipore or Barnstead water system or its equivalent may be used to generate deionized reagent water. Distilled water that has been passed though granular

charcoal may also be suitable. Reagent water is monitored through analysis of the laboratory reagent blank.

7.12 Silicic Acid, ACS grade

7.13 Florisil - 60-100/PR mesh. Activate by heating in a shallow container at 150ºC at least 24 hours and not more than 48 hours.

7.14 Stock Standards - Purchased from Restek or equivalent. The calibration standards must be from a different source than the spiking solution. Stock standards solutions are stored in vials in the organic lab refrigerator until ready to be diluted to the calibration standards. Calibration standards should be checked frequently for signs of evaporation or degradation. Table 1 shows the concentrations of method analytes in the stock and calibration solutions. All analytes in calibration standards must be in the methyl ester form.

7.15 Spiking solution - Purchased from Chemservice or equivalent. The spiking solution must be from a different source than the reference standards. Table 2 shows the concentration of the method analytes in the spiking solution. All analytes in the spiking solution must be in the acid form.

8.0 Sample collection, preservation, and storage

8.1 Grab samples must be collected in glass containers. Conventional sampling practices (3) should be followed; however, the bottle must not be prerinsed with sample before collection.

8.2 Sample preservation and storage

8.2.1 Prior to sample collection, add 80mg sodium sulfite per liter of sample to the collection bottle. The sodium sulfite is added to remove residual chlorine that may be present in the water.

8.2.2 Collect the water sample. DO NOT prerinse the bottle before collecting the sample. DO NOT overfill the bottle with sample, thus washing out the preservatives.

8.2.3 After the sample has been collected, seal and shake vigorously for 1 min to dissolve the preservatives.

8.2.4 The samples must be iced or refrigerated at 4°C away from light from the time of collection until extraction. Preservation study results indicate that analytes present in the samples are stable for 14 days when stored under these conditions. (2)

8.3 Extract Storage

Extracts should be stored at 4°C away from light. Preservation study results indicate that most analytes are stable for 28 days (2); however, it is recommended that they be analyzed within 14 days.

9.0 Quality Control

9.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, determination of surrogate compound recoveries in each sample and blank, analysis of laboratory reagent blanks, laboratory fortified samples, laboratory fortified blanks, and QC samples.

9.2 The analyst is permitted to modify the GC columns, GC conditions, detectors, continuous extraction techniques, concentration techniques, or surrogate compounds. Each time such method modifications are made, the analyst must repeat the procedures in section 9.4.

9.3 Laboratory reagent blanks (LRB) -
Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window of any analyte the LRB produces a peak that would prevent the determination of that analyte, determine the source of the contamination and eliminate the interference before processing samples.

9.4 Initial demonstration of capability.

9.4.1 Select a representative fortified concentration (about 10 times EDL) for each analyte. Prepare a sample concentrate containing each analyte such that when added to a liter of water it will be at the selected concentration. See Table 2 for an example of concentrations. Analyze at least four 1-liter samples at the selected concentration using procedures in section 11.

9.4.2 For each analyte the recovery value for all four of these samples must fall

in the range of R+/-30% (or within R+/- $3S_R$ if broader) using the values for R and S_R for reagent water in Table 3. For those compounds that meet the acceptable criteria, performance is considered acceptable and sample analysis may begin. For those compounds that fail these criteria, this performance must be repeated using five fresh samples until satisfactory performance has been demonstrated.

9.5 Assessing surrogate recovery

9.5.1 When surrogate recovery from a sample or method blank is <70% or >130%, check 1) calculations to locate possible errors, 2) spiking solutions for degradation, 3) contamination, and 4) instrument performance. If those steps do not reveal the cause of the problem, reanalyze the extract.

9.5.2 If a blank extract reanalysis fails the 70-130% recovery criterion, the problem must be identified and corrected before continuing.

9.5.3 If sample extract reanalysis meets the surrogate recovery criterion, report only data for the analyzed extract. If sample extract continues to fail the recovery criterion, report all data for that sample as suspect.

9.6 Assessing Laboratory Performance - Laboratory Fortified Blank (LFB)

9.6.1 The laboratory must analyze at least one LFB sample with every 20 samples or one per set, whichever is the greater frequency. The concentration of each analyte should be 10 times the EDL. Calculate accuracy as percent recovery (X_i). If the recovery of any analyte falls outside the control limits (see section 9.6.2), that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analysis.

9.6.2 Until sufficient data becomes available from within the laboratory, usually a minium of results from 20 to 30 analysis, the laboratory should assess laboratory performance against the control limits in Sect. 9.4.2 that are derived from the data in table 3. When sufficient internal performance data becomes available, develop control limits from the mean percent recovery (X) and standard deviation (S) of the percent recovery. These data are used to establish upper and lower control limits as follows:

Upper Control Limit = X + 3S
Lower Control Limit = X - 3S

After each five to ten new recovery measurements, new control limits

should be calculated using only the most recent 20-30 data points. These calculated control limits should never exceed those established in Section 9.4.2.

9.6.3 It is recommended that the laboratory periodically determine and document its detection limit capabilities for the analysis of interest.

9.6.4 At least quarterly, analyze a QC sample from an outside source.

9.7 Assessing analyte recovery - Laboratory fortified sample matrix

9.7.1 The analyst must add a known concentration to a minimum of 10% of the routine samples or one sample per set, whichever is the greater frequency. The concentration should be the same as that used for the laboratory fortified blank (See sect. 9.6).

9.7.2 Calculate the percent recovery, P of the concentration for each analyte, after correcting the analytical result, X, from the fortified sample for the background concentration, b, measured in the unfortified sample,

$$P = 100(X-b)/ \text{ fortifying concentration},$$

Compare these values to control limits appropriate for reagent water data collected in the same fashion. The appropriate control limits would be the acceptance limits in sect. 9.6.

9.7.3 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (sect. 9.6), the recovery problem encountered with the fortified sample is judged to be matrix related, and not system related. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.8 The laboratory may adopt additional quality control practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of the samples under site conditions, transportation, and storage.

9.9 Tube spike: the tube spike is used to evaluate the effectiveness of the derivatization and to determine if the calibration standards and spike solution match. Results should be within $\pm$ 10%. If not, the problem must be identified

and resolved before performing any analysis of samples.

10.0 Calibration

10.1 Establish GC operating parameters equivalent to those indicated in Sect 6.7. The GC system is calibrated using the external standard technique (sect 10.2). NOTE: Calibration standards solutions must be prepared such that no unresolved analytes are mixed together.

10.2 External Standard Calibration Procedure

10.2.1 Prepare calibration standards at a minimum of three (recommend five) concentration levels for each analyte of interest and surrogate compound by adding volumes of one or more stock standards to a volumetric flask. Dilute to volume with MTBE. NOTE: The analytes must in the methyl ester form for the standards. The lowest concentration should be the EDL, or the reporting level for each analyte. The remaining standards should bracket the analyte concentration expected in the sample extracts, or should define the working range of the detector.

10.2.2 Starting with the standard of lowest concentration, analyze each calibration standard according to sect 11.7 and tabulate response (peak height or area) verses the concentration in the standard. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (calibration factor) is a constant over the working range (20% RSD or less), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve. The chromatography data system software may be used to establish the calibration curve.

10.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (greater than 8 hours), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course of the analysis. If the response for any analyte varies from the predicted response by more than $\pm$20%, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve

or use a single point calibration standard as described in section 10.2.4.

10.2.4 Single point calibration is a viable alternative to a calibration curve. Prepare single point standards from the secondary dilution standards in MTBE. The single point standards should be prepared at a concentration that produces a response that deviates from the sample extract response by no more than 20%.

10.2.5 Verify calibration standards periodically, recommended at least quarterly, by analyzing a standard prepared from reference material obtained from an independent source. Results from these analysis must be within the limits used to routinely check calibration.

11.0 Procedure

11.1 Automated hydrolysis, preparation, and extraction.

Data presented in this method were generated using the automated extraction procedure with the mechanical bottle tumbler.

11.1.1 Add preservative (sect 8.2) to any sample not previously preserved, e.g., blanks and QC check standards. Measure 1L (1000g $\pm$ 5g)of sample into the tumbler bottle.

11.1.2 Add 250g NaCl to the sample, seal, and shake to dissolve salt.

11.1.3 Fortify samples with surrogate. 10uL (125ug/ml) 2,4-DCAA

11.1.4 Fortify the LFB and sample spike with 25uL Herb water spike. See table 2 for a list and concentration of analytes in spiking solution.

11.1.5 Add 20mL of 6N NaOH to the sample, seal and shake. Check the pH of the sample, if the pH is not greater than 12 adjust with more 6N NaOH. Rotate the sample for 1 hour on the mechanical tumbler.

11.1.6 If the sample potentially has a large number of interfering organics proceed with step 11.1.7, if not go on to 11.2.

11.1.7 Add 300 mL methylene chloride to sample. Seal and shake for 10 sec, venting periodically. Repeat shaking and venting until pressure release is

not observed during venting. Reseal and tumble the sample for 1hr. Complete and thorough mixing of the organic and aqueous phases should be observed at least 2 minutes after starting the mixing device.

11.1.8 Remove the sample container from the mixing device. Pour the contents into a 2L separatory funnel. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interference between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration through glass wool, centrifugation, or other physical means. Drain and discard the organic phase. Return the aqueous phase to the tumbler bottle.

11.2 Acidification and extraction

11.2.1 Add 25-30mL of concentrated H_2SO_4 to the sample and mix. Check the pH of the sample with ph paper; if the sample does not have a pH $\leq$ 2, adjust by adding more H_2SO_4.

11.2.2 Add 300mL methylene chloride to the sample, seal, and shake for 10 sec, venting periodically. Repeat shaking and venting until pressure release is not observed during venting. Reseal and tumble for 1 hr. Complete and thorough mixing of the organic and aqueous phases should be observed at least 2 min after starting the mixing.

11.2.3 Pour the contents of the tumbler bottle into a 2L separatory funnel. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third of the volume of the solvent layer, the analyst must employ mechanical techniques to complete the separation. The optimum technique depends on the sample, but may include stirring, filtration, centrifugation, or other physical methods. Collect the organic layer (bottom) into a turbo vap collection vessel.

11.3 Extract Concentration, solvent exchange, and final volume adjustment

11.3.1 Evaporate the extracts to about 1mL using the turbo vap and rinse the inner sides of the turbo vap evaporation vessel with MTBE.

11.3.2 Repeat evaporation and rinsing with MTBE for a total of 3 times.

11.3.3 Quantitatively transfer solvent exchanged extracts to 15mL graduated centrifuge tubes. Evaporate extracts to 1mL under a flow of nitrogen.

11.4 Esterification of acids - See Section 5.2 for safety precautions

11.4.1 Assemble the diazomethane generator (see figure 1) in a hood. The collection vessel is a 10 or 15 mL vial, equipped with a Teflon-lined screw cap and maintained at 0-5°C.

11.4.2 Add a sufficient amount of ethyl ether to tube 1 to cover the first impinger. Add 10mL MTBE to the collection vial. Add 4mL Diazald solution (Sect. 7.8) and 4mL 37% KOH solution (Sect 7.5) to the second tube. Connect tubing as shown and allow the nitrogen flow (5-10mL/min) to purge the diazomethane from the reaction vessel into the collection vial for 30 min or until the color is gone from tube 2. Cap the vial when collection is complete and maintain at 0-5°C. When stored at 0-5°C this diazomethane solution may be used over a period of 48 hrs.

11.4.3 Make up the tube spikes.

11.4.3.1 Add 1mL MTBE to two clean graduated centrifuge tubes.

11.4.3.2 To tube 1, tube spike, add the spiking solution at the same concentration as the samples. See Sect. 11.1.4

11.4.3.3 To tube 2, tube surr, add the surrogate solution at the same concentration as the samples. See Sect. 11.1.3

11.4.4 To each sample in graduated centrifuge tubes add 250uL methanol and 500uL diazomethane solution (sect. 11.4.2). Samples should turn yellow after addition of the diazomethane solution and remain yellow for at least 2 min. Repeat methylation procedure if necessary.

11.4.5 Seal tubes with Teflon lined caps. Mix well and store at room temperature in a hood for at least 30 min.

11.4.6 Destroy any unreacted diazomethane by adding 0.1 to 0.2 grams silicic acid to the centrifuge tubes. Mix well and adjust the sample volume to 5.0mL with MTBE. Allow them to stand until the evolution of nitrogen gas has stopped (approximately 20 min).

11.4.7 If there is sulfur contamination continue on to section 11.5. If no sulfur, transfer about 1mL of sample to autosampler vials and analyze by GC/ECD.

11.5 Florisil Separation - This only needs to be done if there is sulfur contamination in the samples.

11.5.1 Place a small plug of glass wool or filter into a glass drying column. Tare the column and measure 1g of activated Florisil into the column.

11.5.2 Apply 5mL of 5% methanol in MTBE to the Florisil. Allow the liquid to just reach the top of the Florisil. In this and subsequent steps, allow the liquid level to just reach the top of the Florisil before applying the next rinse, however, do not allow the Florisil to go dry. Discard the eluate.

11.5.3 Apply 5mL methylated sample to the Florisil leaving silicic acid in the tube. Collect eluate in a new tube.

11.5.4 Add 1mL of 5% methanol in MTBE to the sample container, rinsing walls. Transfer the rinse to the Florisil column leaving the silicic acid in the tube. Collect eluate in the same tube as 11.5.3. Repeat with 1mL and 3mL aliquots of 5% methanol in MTBE, collecting eluate.

11.5.5 If necessary, evaporate eluate so that final volume is 5mL.

11.5.6 Transfer about 1mL of sample to autosampler vial and analyze by GC/ECD.

11.6 Gas Chromatography

11.6.1 Sect. 6.7 summarizes the recommended operating conditions for the GC. Included in table 4 are retention times observed using this method. Other GC columns, chromatographic conditions, or detectors may be used.

11.6.2 Calibrate the system daily as described in Sect. 10.0. The standards and extracts must be in MTBE.

11.6.3 Inject 2uL of the sample extract. Record the resulting peak size in area units and the retention times of the peaks. The chromatography data system may be used to record data.

11.6.4 If the response for the peak exceeds the response for the highest concentration calibration standard, dilute the extract and reanalyze.

11.7 Identification of analytes

11.7.1 Identify a sample component by comparison of its retention time to the retention times of the components in the standard chromatogram. If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound, then identification is considered positive.

11.7.2 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.7.3 True identification of the analytes requires confirmation on a dissimilar column (see section 6.7). When analytes appear to not be resolved chromatographically on the two columns the GC/MS may be used to positively identify the components of the sample.

12.0 Calculations

12.1 Calculate analyte concentrations in the sample from the response for the analyte using the calibration procedure described in Sect. 10.0.

12.2 The chromatography data system software may be used to establish the calibration curve and calculate the concentration of the analytes in each sample. If the data system is not used the calculations are found is section 12.3.

12.3 Calculate the amount of material injected from the peak response using the calibration curve or calibration factor determined in section 10.2. The concentration (C) in the sample can be calculated from the following equation:

$$C(ug/L) = (A)(V_t) / (V_i)(V_s)$$

Where:

A = Amount of material injected (ng)
V_i = Volume of extract injected (uL)
V_t = Volume of total extract (uL)

V_s = Volume of water extracted (mL)

13.0 Method Performance

In this laboratory, analyte recoveries from 10 replicate fortified reagent water samples were determined. Results were used to determine analyte EDLs and are summarized in Table 5.

14.0 Pollution Prevention

The large volume of organic solvent is a potential for pollution. The analyst should take care to properly use the solvent in vented hoods. The excess solvent can be placed in hoods to evaporate or can be recycled or disposed of in an environmentally sound manner according to local regulations.

15.0 Waste Management

15.1 The volume of organic solvent should have a plan of action for waste management. The water samples can be diluted and put down the drain. The organic solvent can be recycled or evaporated into a vented hood.

15.2 For further information on waste management consult The Waste Management Manual for Laboratory Personnel and Less is Better: Laboratory Chemical Management for Waste Reduction, both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

16.0 References

1. "Determination of Chlorinated Acids in Water by Gas Chromatography with an Electron Capture Detector" EPA Method 515.1, Revision 4.0. R.C. Dressman et al.

2. National Pesticide Survey Method No. 3, "Determination of Chlorinated Acids in Water by Gas Chromatography with an Electron Capture Detector."

3. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

4. Code of Federal Regulations, Title 40, Part 136, Appendix B. Published by the Federal Register National Archives and Records Administration.

17.0 Tables and Figures

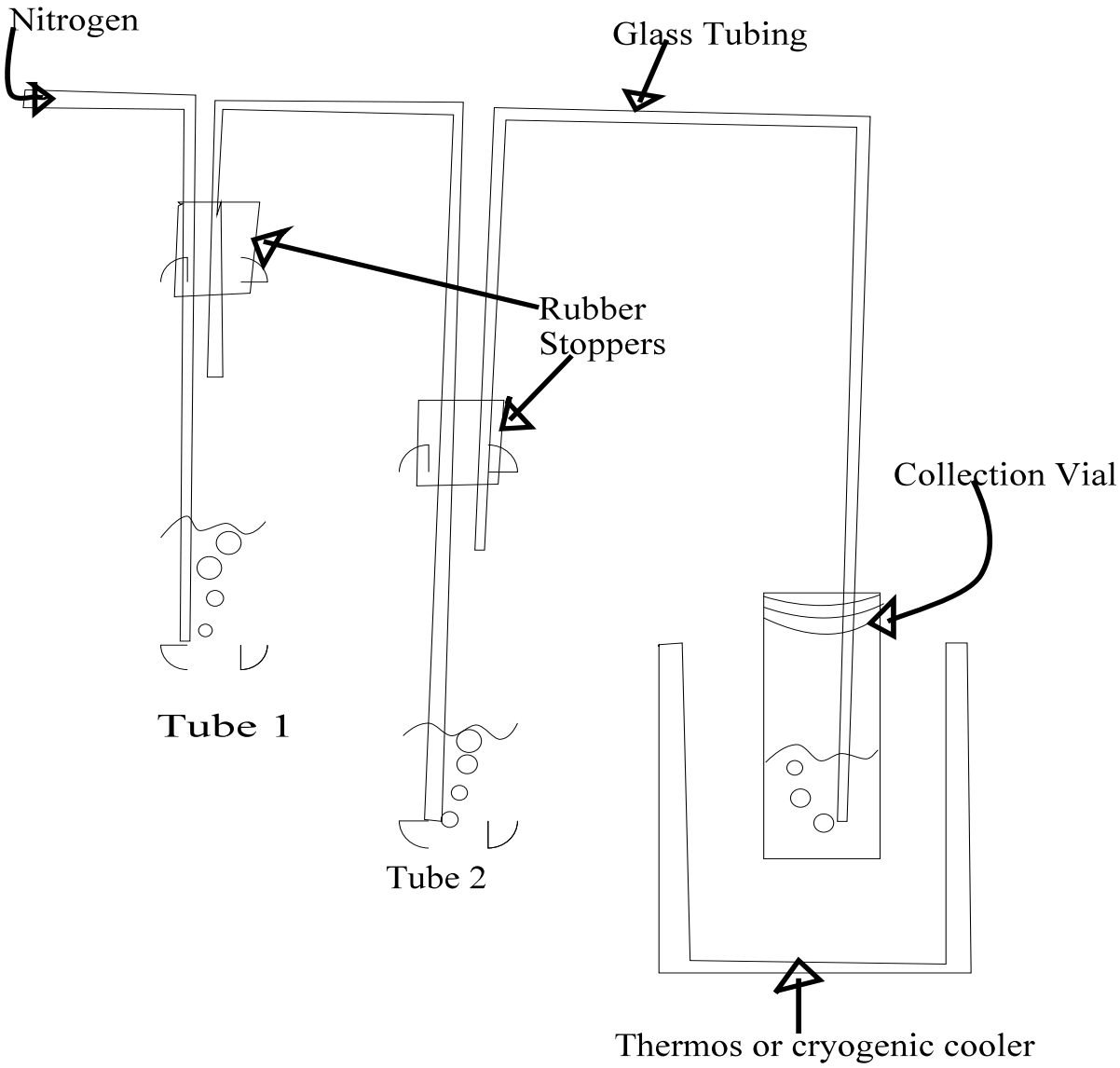

Figure 1: Diazomethane Solution Generator

Table 1: Calibration Standards

Concentration for each analyte in Calibration Standards
All analytes are in the methyl ester form
The amount of stock solution diluted into 10mL of MTBE is listed for each standard

Analyte	Stock Solution	Standard 1 (MDL) 20uL	Standard 2 40uL	Standard 3 80uL	Standard 4 200uL	Standard 5 800uL
Dalapon	25ug/mL	0.05ug/mL	0.1ug/mL	0.2ug/mL	0.5ug/mL	2.0ug/mL
3,5-DCBA	12.5	0.025	0.05	0.1	0.25	1.0
2,4-DCAA	25.0	0.05	0.1	0.2	0.5	2.0
Dicamba	5.0	0.01	0.02	0.04	0.1	0.4
MCPA	1250.0	2.5	5.0	10.0	25.0	100.0
Dichlorprop	15.0	0.03	0.06	0.12	0.3	1.2
2,4-D	10.0	0.02	0.04	0.08	0.2	0.8
Bromoxynil	2.5	0.005	0.01	0.02	0.05	0.2
PCP	2.0	0.004	0.008	0.016	0.04	0.16
2,4,5-TP	5.0	0.01	0.02	0.04	0.1	0.4
2,4,5-T	5.0	0.01	0.02	0.04	0.1	0.4
Dinoseb	10.0	0.02	0.04	0.08	0.2	0.8
Bentazon	25.0	0.05	0.1	0.2	0.5	2.0
Dacthal	2.5	0.005	0.01	0.02	0.05	0.2
Picloram	5.0	0.01	0.02	0.04	0.1	0.4
Acifluorfen	10.0	0.02	0.04	0.08	0.2	0.8
Diclofop	25.0	0.05	0.1	0.2	0.5	2.0

Table 2: Spiking Solution Concentration

Concentration of analytes in spiking solution
All analytes must be in the acid form
Solvent: MTBE

Analyte	Concentration
Dalapon	50 ug/mL
3,5-DCBA	25
Dicamba	10
MCPA	2500
Dichlorprop	30
2,4-D	20
Bromoxynil	5
PCP	4
2,4,5-TP	10
2,4,5-T	10
Dinoseb	20
Bentazon	50
Dacthal	5
Tordon	10
Acifluorfen	20
Hoelon	50

Table 3: Upper and Lower Control Limits

The percent recovery the analytes in the LFB, LRB, and spike must be between in order to pass for the run.

Analyte	Lower Limit	Upper Limit
3,5-DCBA	53.1%	150.9%
2,4-DCAA	70.0%	130.0%
Dicamba	37.8%	232.4%
MCPA	NA	NA
Dichlorprop	46.1%	167.9%
2,4-D	48.5%	213.5%
Bromoxynil	NA	NA
PCP	36.4%	223.6%
2,4,5-TP	41.6%	226.4%
2,4,5-T	67.8%	166.2%
Dinoseb	-0.9%	84.9%
Bentazon	69.6%	170.4%
Dacthal	NA	NA
Tordon	44.5%	137.5%
Acifluorfen	73.9%	168.1%
Hoelon	NA	NA

Table 4: Guide to retention times

Retention time and order found on the columns
NOTE: These time are meant to be a guide only

Analyte	Primary Column RTX-50ms	Confirmation Column RTX-5ms
Dalapon	3.24	2.74
3,5-DCBA	12.04	10.70
2,4-DCAA	14.18	12.27
Dicamba	14.57	12.47
MCPA	15.12	13.06
Dichlorprop	15.50	13.66
2,4-D	16.18	13.94
Bromoxynil	16.43	13.87
PCP	16.78	15.00
2,4,5-TP	17.12	15.36
2,4,5-T	17.88	15.70
Dinoseb	18.24	16.47
Dacthal	20.30	17.99
Tordon	21.63	17.42
Acifluorfen	25.01	20.70
Hoelon	31.72	23.26

Injector @ 235ºC, Detectors @ 300ºC
Oven Program: Hold at 50ºC for 1 minute
Ramp to 226.0ºC at 10C/min, hold 12 minutes
Ramp to 286.0ºC at 15C/min, hold 10 minutes

Table 5: Analyte Accuracy and Precision Data[1]

Analyte	Fortified Conc ug/L	Mean Measured Conc ug/L	Std Dev ug/L %	EDL[2] ug/L
3,5-DCBA	0.63	0.57	7.1	0.125
2,4-DCAA	1.25	1.38	20	0.25
Dicamba	0.25	0.25	3.4	0.05
MCPA	62.50	53.72	530	12.5
Dichlorprop	0.75	0.74	9.0	0.15
2,4-D	0.50	0.51	5.7	0.10
Bromoxynil	0.13	0.12	1.7	0.025
PCP	0.10	0.09	1.4	0.02
2,4,5-TP	0.25	0.26	2.7	0.05
2,4,5-T	0.25	0.26	2.9	0.05
Dinoseb	0.50	0.46	8.6	0.10
Bentazon	1.25	1.20	12	0.25
Dacthal	0.13	0.10	1.6	0.025
Tordon	0.25	0.19	3.9	0.05
Acifluorfen	0.50	0.53	16	0.1
Hoelon	1.38	1.34	15	0.25

[1]Produced by analysis of 10 fortified water samples
[2]Estimated Detection Limit/Standard 1 level